EXHIBIT 30

TOXICOLOGICAL PROFILE FOR NICKEL

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Public Health Service
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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO NICKEL IN THE UNITED STATES

Nickel is a very hard metal that occurs naturally in soils and volcanic dust. Nickel is used in combination with other metals to form alloys used for coins, jewelry, and stainless steel. Nickel compounds are used for electroplating, to color ceramics, and in battery production.

Nickel is released to the atmosphere by windblown dust, volcanoes, combustion of fuel oil, municipal incineration, and industries involved in nickel refining, steel production, and other nickel alloy production. The form of nickel emitted to the atmosphere is dependent upon the source. Complex nickel oxides, nickel sulfate, and metallic nickel are associated with combustion, incineration, and smelting and refining processes. Ambient air concentrations of nickel range between 7 and 12 ng/m^3, mainly in the form of aerosols and can be as high as 150 ng/m^3 near point sources. Based on 1996 air quality data, EPA has reported average U.S. ambient air levels of 2.2 ng/m^3. Ambient air levels of nickel are expected to be higher in urban air than in rural air. Concentrations of nickel in indoor air are generally 10 ng/m^3.

Background levels of nickel in soils vary widely depending on local geology and anthropogenic inputs, but concentrations typically range between 4 and 80 ppm. Some areas of the United States may contain natural levels as high as 5,000 ppm. Concentrations of nickel in household dust can be high and therefore pose an increased risk to young children who have greater contact with floors. Nickel concentrations in surface water and groundwater range between 3 and 10 µg/L. Nickel levels in drinking water in the United States generally range from 0.55 to 25 µg/L and average between 2 and 4.3 µg/L. Based on these average nickel concentrations and a reference water intake of 2 L/day, the estimated average intake of nickel from drinking water ranges from 4 to 8.6 µg/day. Elevated levels of nickel may exist as a result of the corrosion and leaching of nickel alloys used in valves and faucets. For the general population, the predominant route of exposure to nickel is through food intake. Nickel intake in the United States ranges between 69 and 162 µg/day for adults (>18 years of age). Based on these average water and food nickel levels, a daily dose of 0.001–0.0024 mg/kg/day can be estimated using a reference body weight of 70 kg. In children, mean daily nickel intakes of 9, 39, 82, and 99 µg/day have been determined for children aged 0–6 months, 7–12 months, 1–3 years, and 4–8 years, respectively. The mean daily dietary intakes of

nickel in children aged 9–18 years (128–137 µg/day in males and 101–109 µg/day for females) are similar to the mean intakes determined in adults (>18 years of age).

A 70 kg reference man contains 10 mg of nickel, giving an average body concentration of 0.1 ppm. Reference values for nickel in healthy adults is 0.2 µg/L in serum and 1–3 µg/L in urine. A National Health and Nutritional Examination Survey II of hair found mean nickel levels of 0.39 ppm, with 10% of the population having levels >1.50 ppm.

About 20–35% of the inhaled nickel that is retained in the lungs is absorbed into the blood. Absorption of nickel following oral exposure has been shown to vary (3–40%) depending on whether the nickel was in drinking water or food, with greater absorption occurring with drinking water. Fasting individuals have also been shown to absorb more nickel from the gastrointestinal tract. Most of the absorbed nickel is excreted in the urine, regardless of the route of exposure.

Nickel does not bioaccumulate to a great extent in animals. There is evidence of uptake and accumulation in certain plants.

Nickel is an essential trace element in animals, although the functional importance of nickel has not been clearly demonstrated. It is considered essential based on reports of nickel deficiency in several animal species (e.g., rats, chicks, cows, goats). Nickel deficiency is manifested primarily in the liver; effects include abnormal cellular morphology, oxidative metabolism, and increases and decreases in lipid levels. Decreases in growth and hemoglobin concentration and impaired glucose metabolism have also been observed. The essentiality of nickel in humans has not been established, and nickel dietary recommendations have not been established for humans.

2.2 SUMMARY OF HEALTH EFFECTS

The general population can be exposed to nickel via inhalation, oral, and dermal routes of exposure. Based on occupational exposure studies, reports of allergic contact dermatitis, and animal exposure studies, the primary targets of toxicity appear to be the respiratory tract following inhalation exposure, the immune system following inhalation, oral, or dermal exposure, and possibly the reproductive system and the developing organism following oral exposure.

Oskarsson 1991). An *in vitro* study of rat hepatocytes found that the calcium channels are involved in nickel uptake by the liver (Funakoshi et al. 1997). At physiological levels, no tissue significantly accumulates orally administered nickel (Nielsen 1990).

Nickel that is absorbed is excreted primarily in the urine. In the urine, nickel is primarily associated with low molecular weight complexes that have free amino acids as indicated by the ninhydrin reaction (Sunderman and Oskarsson 1991). In humans nickel is also eliminated in hair, skin, milk, and sweat.

The physiological role of nickel in animals and humans has not yet been identified. The most likely roles are as cofactors in metalloenzymes or metalloproteins, or as a cofactor that facilitates the intestinal absorption of iron (Fe^{3+} ion) (Nielsen 1982). Support for a role of nickel in enzymes comes from the identification of nickel-containing enzymes in plants and microorganisms. The types of nickel-containing enzymes that have been identified are urease, hydrogenase, methylcoenzyme M reductase, and carbon monoxide dehydrogenase (Nielsen 1990). Nickel may also have a role in endocrine gland function as suggested by its effect on prolactin levels.

3.5.2 Mechanisms of Toxicity

The mechanism of adverse respiratory effects following lung exposure of rabbits to metallic nickel or nickel chloride has been examined (Johansson and Camner 1986; Johansson et al. 1980, 1981, 1983, 1987, 1988a, 1989). In these studies, an accumulation of macrophages and granular material (primarily phospholipids) in the alveoli and an increase in volume density of alveolar type II cells were observed. The type II cells contained large amounts of lamellar bodies. Similar results were found following exposure to metallic nickel and nickel chloride, indicating that nickel ions apparently had a direct effect on type II cells (Johansson and Camner 1986). At the end of 6 months, all of the rabbits had foci of pneumonia, indicating an increased susceptibility to infection (Johansson et al. 1981). This may have been a result of the decreased function of the alveolar macrophages.

The substitution of nickel for other essential elements may also contribute to the adverse effects of nickel. Nickel can replace magnesium in certain steps in the activation of complement (McCoy and Kenney 1992). For example, the replacement of nickel for magnesium can increase the formation of C3b, Bb enzyme by 40 times, which amplifies activation of the complement pathway. Nickel has also been shown to activate calcineurin, a phosphatase that binds zinc and iron, and is usually activated by manganese.

There is some evidence that nickel may have a role in the release of prolactin from the pituitary. *In vitro* studies have shown that nickel could directly inhibit the release of prolactin by the pituitary, and it has been suggested that nickel may be part of a prolactin inhibiting factor (LaBella et al. 1973). Intravenous exposure to nickel chloride has been shown to reduce serum levels of prolactin in male rats that were pretreated with chlorpromazine, which itself produces hyperprolactinemia (LaBella et al. 1973). The effect was not observed in rats that had not been pretreated with chlorpromazine. Nickel has also been shown to accumulate more in the pituitaries of pregnant rats than nonpregnant rats (Sunderman et al. 1978), suggesting that a toxicological effect through prolactin may only be manifested during maximum prolactin production. A subcutaneous injection study has also shown that nickel can change the quality of the milk produced, resulting in increased milk solids (42%) and lipids (110%), and decreased protein (29%) and lactose (61%) (Dostal et al. 1989). Because these changes were noted in comparison to pair-fed rats, they were not considered to be a result of changes in food intake.

The mechanism of nickel carcinogenicity has not been firmly established; it is likely that the carcinogenic effects result from a variety of mechanisms. The available evidence suggests that, mechanistically, nickel carcinogenicity is probably the result of genetic factors and/or direct (e.g., conformational changes) or indirect (e.g., generation of oxygen radicals) epigenetic factors. Additionally, certain nickel compounds promote cell proliferation, which would convert repairable DNA lesions into nonrepairable mutations. Nickel is considered to be genotoxic, but has a low mutagenic potential (Kasprzak et al. 2003b). The nickel-induced DNA damage has resulted in the formation of chromosomal aberrations (Conway and Costa 1989; Dhir et al. 1991; Larramendy et al. 1981; Lechner et al. 1984; Sen and Costa 1986b; Sen et al. 1987; Waksvcik and Boysen 1982) that could result in deletion of senescence or tumor suppressor genes. Nickel compounds have also been found to be weak inducers of sister chromatid exchanges (Andersen 1983; Arrouijal et al. 1992; Larramendy et al. 1981; Ohno et al. 1982; Saxholm et al. 1981; Wulf 1980).

Although nickel has a relatively weak affinity for DNA, it has a high affinity for chromatin proteins, particularly histones and protamines (Costa et al. 1994; Kasprzak et al. 2003b; Oller et al. 1997). The complexing of nickel ions with heterochromatin results in a number of alterations including condensation, DNA hypermethylation, gene silencing, and inhibition of histone acetylation. These alterations have been shown to disturb gene expression (Costa et al. 1994; Kasprzak et al. 2003b; Lee et al. 1995; Oller et al. 1997; Zoroddu et al. 2002). Methylation of DNA may result in critical genes becoming incorporated into heterochromatin where they can no longer be expressed (Costa 1995). Some of the alterations in gene expression may be mediated by activated transcription factors. Nickel has been shown to alter several

transcription factors including hypoxia-inducible transcription factor (HIF-1), activating transcription factor (ATF-1) involved in inactivation of thrombospondin-1, which suppresses angiogenesis, and NF-$_K$B transcription factor involved in the inducible expression of adhesion molecules (Kasprzak et al. 2003b). The strongest epigenetic effects on nickel have been associated with HIF-1. The HIF-1 transcription factor is involved in the regulation of hypoxia-inducible genes involved in cell transformation, tumor promotion, and progression, angiogenesis, altered metabolism, and apoptosis. HIF-1α, one of the HIF-1 subunits, is over-expressed in both primary and metastatic tumors. It is induced in response to hypoxia and exposure to nickel (Li et al. 2004; Salnikow et al. 2000b). Both soluble and insoluble nickel compounds have also been shown to induce Cap43 (also called NDRG2) gene expression, which requires HIF-1α activation (Costa et al. 2003; Li et al. 2004; Salnikow et al. 2000b). There is also evidence that nickel ions inhibit DNA repair (Hartwig et al. 1994). Nickel enhances the genotoxicity of ultraviolet light, x-rays, *cis*- and *trans*-platinum, and mitomycin C. *In vitro* studies in HeLa cells suggest that nickel inhibits the incision step in excision repair (Hartwig et al. 1994), while studies using Chinese hamster ovary cells suggest that nickel inhibits the ligation step of excision repair (Lee-Chen et al. 1994). The underlying mechanism of how nickel affects DNA repair is unclear. Sunderman and Barber (1988), Sunderman (1989b), and Hartwig et al. (1994) suggest that nickel ions may compete with zinc ions for binding to zinc-finger DNA binding proteins, resulting in structural changes in DNA that prevent repair enzymes from binding. Nickel may also directly interact with enzymes required for DNA repair (Hartwig et al. 1994).

The binding of nickel to the histone protein within heterochromatin could result in the generation of oxygen radicals. These oxygen radicals could subsequently induce damage bases, DNA strand breaks, and DNA protein crosslinks (Costa et al. 1994; Oller et al. 1997). The available evidence suggests that this mechanism would play a minor (if any) role in nickel carcinogenicity because the damage would be confined to heterochromatin regions of DNA, which lack active genes (Oller et al. 1997). However, nickel ions can complex with a number of cellular ligands including amino acids, peptides, and proteins resulting in the generation of oxygen radicals. The reactive oxygen species (ROS) generated could nonselectively damage DNA, possibly resulting in genetic changes in active genes (Kasprzak et al. 2003b; Oller et al. 1997).

3.5.3 Animal-to-Human Extrapolations

The available data on the toxicity of inhaled nickel provide strong evidence that the respiratory tract, in particular the lung, is the most sensitive target of nickel toxicity in humans and animals. There are